

*National Toxicology Program  
Interagency Center for the Evaluation of  
Alternative Toxicological Methods*

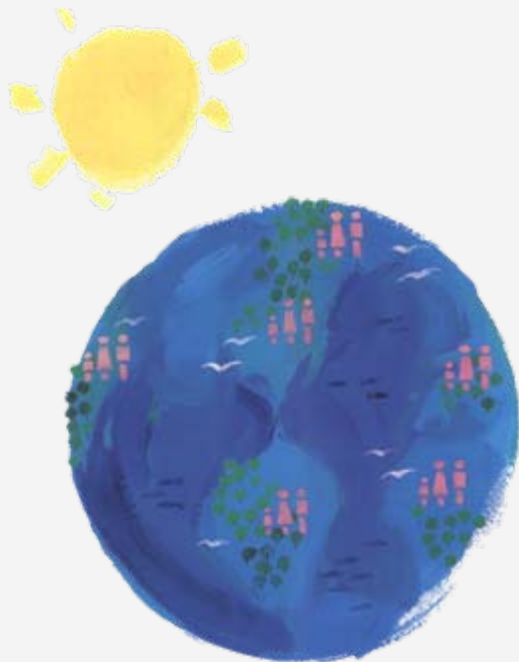
*Interagency Coordinating Committee on  
the Validation of Alternative Methods*

## Workshop on Acute Chemical Safety Testing: Advancing *In Vitro* Approaches and Humane Endpoints for Systemic Toxicity Evaluations

### II. Workshop Recommendations

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# Workshop Breakout Groups

- *Breakout Group 1:*

Key Pathways for Acute Systemic Toxicity

- *Breakout Group 2:*

Current Acute Systemic Toxicity Injury and Toxicity Assessments

- *Breakout Group 3:*

Identifying Earlier Humane Endpoints for Acute Systemic Toxicity Testing

- *Breakout Group 4:*

Application of *In Vivo* Mode of Action and Mechanistic Information to the Development and Validation of *In Vitro* Methods for Assessing Acute Systemic Toxicity

- *Breakout Group 5:*

Industry Involvement in Test Method Development, Validation, and Use



# Breakout Group 1: Key Pathways for Acute Systemic Toxicity

Co-chairs: Drs. Daniel Acosta and Frank Paloucek

## Objectives

1. Discuss current understanding of key pathways for *in vivo* acute systemic toxicity;
  - identify knowledge gaps that exist, especially for
    - (a) *In vivo* pathways, and
    - (b) Various chemicals and products (*Workshop Objective 1*)
2. Identify and prioritize future research initiatives
  - to address these knowledge gaps
  - necessary to advance development and validation of *in vitro* methods for assessing acute systemic toxicity. (*Workshop Objective 2*)
3. Review molecular, cellular, tissue, physiological, and clinical biomarkers that are or could be measured or observed during *in vivo* acute systemic toxicity testing
  - discuss their potential usefulness for indicating key pathways of acute systemic toxicity. (*Workshop Objective 3*)

# Breakout Group 1:

## Conclusions and Recommendations (1)

- Key pathways that should be further studied to better understand the toxic effects of chemicals and to better understand and treat acute human poisonings:
  - General cellular function
  - Neuronal transmission, both central and peripheral
  - Sodium/potassium ATP-ase pump
  - Xenobiotic metabolism
  - Cardiac conduction and aerobic metabolism
  - Oxidative stress
  - Receptor activity
  - Immune response and function

# Breakout Group 1:

## Conclusions and Recommendations (2)

- Knowledge gaps related to the diagnosis and treatment of human poisonings
  - Definitive identification of the class of toxicant ingested
  - Toxicant/serum concentration vs. time exposure data
  - Accuracy of patient history reports
  - Laboratory confirmation of known toxicant from reported cases
  - Time course of acute life-threatening poisonings
  - Chemical interactions (e.g., mixtures, polypharmacy, food additives)
- Toxicological observations and measurements to address these gaps. For example:
  - Biomarkers of organ/system damage
  - Markers of oxidative stress

# Breakout Group 1:

## Conclusions and Recommendations (3)

- Recommended research and development activities:
  - High priority:
    - Mode of action-based test methods
    - Human cell-based systems
    - High throughput screening initiatives, computational toxicology, and associated data management capabilities
  - Lower priority (but still particularly important)
    - Determination of *in vitro* and *in vivo* toxicokinetic information
    - Methods to evaluate recovery/reversibility of effects
    - Methods capable of evaluating of organic/hydrophobic materials

# Breakout Group 2: Current Acute Systemic Toxicity Injury and Toxicity Assessments

Co-chairs: Dr. A. Wallace Hayes and Dr. Daniel Marsman

## Objectives

1. Discuss and identify observations and quantitative, objective measurements that could or should be included in current *in vivo* acute systemic toxicity tests to elucidate key toxicity pathways that would support the future development and validation of predictive *in vitro* methods.  
(Workshop Objective 5)

# Breakout Group 2:

## Conclusions and Recommendations (1)

- Biomarkers (clinical observations and quantitative measurements) expected to provide more information and a better understanding of the pathophysiological effects and modes/mechanisms of acute systemic toxicity in current animal tests:
  - Non-invasive or minimally invasive methods should be developed for collecting additional biomarker measurements to maximize the use of the limited number of animals currently required for acute toxicity tests.
  - Early time points after dosing are best for biomarker studies (less than 24 hours).
  - Given the vast number of potential blood/serum biomarkers and the relatively small blood/serum volume available from a rat, there is also a need for continued protocol refinement to reduce the required sample volume and increase the number of tests that can be performed.
  - Target tissues should be properly stored for future research and development studies related to new biomarkers (e.g., those identified using "-omics" technologies).

# Breakout Group 2:

## Conclusions and Recommendations (2)

- Activities for obtaining more information on key toxicity pathways from the current *in vivo* acute systemic toxicity tests
  - Short-Term Activities
    - Standardize procedures for sample collection and processing, as well as biomarker detection and quantification
    - Investigate noninvasive data collection devices/procedures for obtaining detailed physiological data in animal studies
    - Develop biomarkers for pathophysiological effects and modes/mechanisms of acute systemic toxicity
    - Develop alternative test systems to model key pathways for whole animal system, whole organs, and cellular systems
  - Long-Term Activities
    - Use "-omics" technologies to identify sensitive biomarkers (e.g., metabolomics)
    - Imaging: pursue non-invasive technique such as ultrasound or other imaging techniques
    - Nanotechnology (e.g., for use in early detection of toxicity)

# Breakout Group 3: Identifying Earlier Humane Endpoints for Acute Systemic Toxicity Testing

Co-chairs: Dr. Helen Diggs and Dr. Steven Niemi

## Objectives

1. Discuss what *in vivo* data collected to elucidate key toxicity pathways might lead to the identification and validation of more humane endpoints for acute systemic toxicity testing, and what data should be a priority for collection to aid in identifying earlier more humane endpoints. (*Workshop Objective 7*)

# Breakout Group 3:

## Conclusions and Recommendations (1)

- Use of Biomarkers to Identify Earlier Humane Endpoints for Acute Toxicity Tests
  - Focused on identifying *in vivo* data collected to elucidate key toxicity pathways that might lead to the identification and validation of earlier more humane endpoints for acute systemic toxicity testing
  - Discussed the concept of using evident toxicity as an earlier more humane endpoint rather than moribund condition or death
  - Recommended the Fixed Dose Procedure (FDP) as the preferred acute oral toxicity test method for routine use, unless adequate scientific justification provided for use of one of the other two alternative methods (Up and Down Procedure [UDP] and Acute Toxic Class method [ATC])
    - Evident toxicity minimizes or avoids the use of moribund condition or death as an endpoint, which are the endpoints for the UDP and ATC methods
    - Recognized that the FDP may not provide the desired information for some regulatory agencies as does the UDP (i.e., FDP and ATC provide LD50 estimates in a range rather than a point estimate with confidence limits)
    - Need objective criteria to characterize evident toxicity and internationally harmonized guidance to support wider use of the FDP
    - OECD TG 420 is not equivalent to OECD TG 423 and OECD TG 425. The decision criteria in the FDP test guideline include death along with evident toxicity. OECD TG 420 introduced an animal welfare override at every initial test dose allowing for classification based on the outcome for a single animal. Some workshop participants stated that this component of the FDP was not validated.

# Breakout Group 3:

## Conclusions and Recommendations (2)

- Recognized the need to develop two globally standardized scoring systems that allow for weighting of observations
  - Evident toxicity scoring system
  - Severe toxicity/lethality scoring system
- Recommended applying the fixed dose/fixed concentration approach for dermal and inhalation acute toxicity studies in order to use evident toxicity as an earlier more human endpoint for such studies
  - Some U.S. regulatory agency representatives at the workshop did not agree that the FDP should be the preferred method for any acute systemic toxicity testing, including potential applications to acute dermal toxicity and acute inhalation toxicity. Recommendations for using the FDP were made only in the context of identifying humane endpoints; there are scientific and regulatory reasons for using a method other than the FDP. The UDP for acute oral toxicity was developed to provide LD<sub>50</sub> values to satisfy U.S. regulatory requirements.
- Biomarkers considered sufficiently predictive as humane endpoints that they should now be used routinely during acute toxicity testing:
  - Simple behavioral observations for evaluating level of activity
  - Body temperature decreases
  - Body weight changes
  - Changes in feed and water consumption/hydration status
- Recommended routine observation and recording of clinical signs of pain and distress
- Identified several types of data to collect during future animal studies because these data could aid in identifying earlier more humane endpoints

# Breakout Group 3:

## Conclusions and Recommendations (3)

- More objective measurements are needed vs. traditional subjective evaluations
  - Objective measurements would facilitate collection and interpretation of mechanistic data
  - The use of earlier endpoints would avoid “found dead” animals and autolyzed tissues
  - Systematic collection of standardized data will aid in assessing potential earlier endpoints
- Research, Development, and Validation Activities: Humane Endpoints
  - R&D and validation efforts should address knowledge gaps currently associated with predictive early humane endpoints
  - Develop objective criteria with to characterize evident toxicity
    - Publish internationally harmonized guidance on these criteria before initiating routine use of the FDP

# Breakout Group 3:

## Conclusions and Recommendations (4)

- Implementation of Recommended Activities: Humane Endpoints
  - Emphasized the importance of approaches to data mining and sharing of information among international stakeholders to make use of existing and newly generated data, where possible
  - Emphasized the need for opportunities for additional training towards applying the recommended measurements and observations, as well as interpreting their results
  - Dedicated funding is central to future progress
  - Recognized need for other incentives to motivate stakeholders to pursue these activities
  - Well-defined strategies will facilitate implementation

# Breakout Group 4: Application of *In Vivo* Mode of Action and Mechanistic Information to the Development and Validation of *In Vitro* Methods for Assessing Acute Systemic Toxicity

Co-chairs: Drs. Melvin Andersen and Eugene Elmore

## Objectives

1. Discuss how key toxicity pathways indicated by *in vivo* measurements (molecular, cellular, tissue, or other physiological, and clinical biomarkers [see *Workshop Objective 3*]) and observations are currently or could be modeled using alternative *in vitro* test methods. (*Workshop Objective 4*)
2. Identify and prioritize research, development, and validation activities for *in vitro* test methods that model the key *in vivo* toxicity pathways and more accurately predict acute systemic toxicity hazard categories. (*Workshop Objective 6*)



# Breakout Group 4:

## Conclusions and Recommendations (1)

- *In Vivo* Toxicity Pathways to Be Modeled by *In Vitro* Systems
  - Integrated batteries of test methods will be useful to identify pathways
    - e.g., *in vitro* basal cytotoxicity, direct cytotoxicity, apoptosis, cell proliferation
  - Using *in vitro* tests to assess interactions among tissues presents a challenge. Such interactions include:
    - Activation of inflammatory responses following toxicity in one organ leading to enhanced target organ toxicity or in remote tissue targets
    - Interactions between initial target tissues and immune system components.
  - High throughput test methods could be used to identify cellular targets of test chemicals and *in vitro* cell models of these targets could be developed for routine application
  - Use genomic and other “-omic” approaches for inferring both cellular and tissue level pathways that are altered in tissues within animals undergoing acute systemic toxicity testing

# Breakout Group 4:

## Conclusions and Recommendations (2)

### ■ *In Vitro* Modeling of *In Vivo* Acute Systemic Toxicity

- Long-term goals
  - Identify initial cellular pathways (e.g., oxidative stress, loss of membrane function, specific interaction with key receptors)
  - Develop quantitative modeling of the integrated cellular and tissue cascades that follow from these initial interactions leading to acute systemic toxicity.
  - Dose-response models for predicting acute systemic toxicity based on the patterns and dose-response characteristics of pathways perturbed by chemical treatment *in vitro*
- Use *in vitro* test methods to evaluate toxicity pathways to access both specific endpoints and dose-response characteristics
  - Integrated cell responses (e.g., cellular glutathione concentration, mitochondrial function, cytotoxicity, apoptosis, proliferation)
  - Interaction with cellular targets, such as over-expression of transporters in cell lines and examination of uptake rates of chemical into these cells

# Breakout Group 4:

## Conclusions and Recommendations (3)

- Knowledge Gaps Related to *In Vitro* Modeling of *In Vivo* Acute Systemic Toxicity
  - Major knowledge gap is in understanding all of the *in vivo* mechanisms of action of chemicals that would then help direct selection of *in vitro* test method systems
  - There is little experience in assessing correlations between LD<sub>50</sub> and integrated cellular responses other than the basal cytotoxicity test methods
  - No quantitative procedures have been developed to describe cascades of responses and predict LD<sub>50</sub>
  - Use of human based *in vitro* cell systems is a priority to address data requirements that would allow an *in vitro* approach to predicting target tissues and the LD<sub>50</sub> for acute toxicity

# Breakout Group 4:

## Conclusions and Recommendations (4)

### ■ Recommended Research, Development, and Validation Activities

- Collect standardized information from *in vivo* studies conducted for regulatory purposes to better understand modes of action and use this information to guide selection of *in vitro* test methods
- Identify/develop tissue specific cellular models to assess the critical toxicity pathways; incorporate genetic variability
- Apply a broad array of *in vitro* test methods to screen for modes of action
- Convene a meeting of experts in each of the associated target tissues and their cellular pathways to address the development and validation of *in vitro* test methods for measuring cellular response pathways underlying toxic responses
- Convene expert panels to address the issues of development of cell lines, designing and using appropriate biomarkers, test method implementation, and data analysis procedures

# Breakout Group 4:

## Conclusions and Recommendations (5)

- Recommended Research, Development, and Validation Activities (cont)
  - Use human cord blood to isolate human stem cells and to direct their differentiation to express the biomarkers normally expressed in the tissue of interest
  - Select and use test chemicals that are active in the toxic response pathway as well as negative controls
  - Develop databases of genomic changes and assess affected tissue level pathways in animals undergoing acute systemic toxicity testing
  - Develop computational systems biology approaches to predict *in vivo* acute toxicity from sequential activation of specific cellular pathways

# Breakout Group 4:

## Conclusions and Recommendations (6)

### ■ Implementation of Recommended Activities

- Identify model cellular systems for assessing chemical activity in the pathway and identify agents that relate to toxicity in these systems
- Interpret results using standardized test panels to compare with the rodent LD<sub>50</sub>
- Use statistical tools currently being developed and implemented to facilitate interpretation for association between potency in specific pathway test methods and the rodent LD<sub>50</sub>
- Determine the effectiveness of each system alone and in combination to predict *in vivo* toxicity
- Consider incorporating these individual test methods into the assessment of acute toxicity in parallel to the *in vitro* basal cytotoxicity test method as currently used
- Develop appropriate analysis procedures to compare performance of new test methods in relation to the *in vitro* basal cytotoxicity test methods for predicting the rodent LD<sub>50</sub>

# Breakout Group 5: Industry Involvement in Test Method Development, Validation, and Use

Co-chairs: Dr. Robert Scala and Dr. William Stott

## Objectives

1. Discuss how to promote the collection and submission of *in vitro* and *in vivo* toxicity test data to ICCVAM in order to advance the development and validation of more predictive *in vitro* test methods and earlier more humane endpoints for acute systemic toxicity testing. (*Workshop Objective 8*)

# Breakout Group 5:

## Conclusions and Recommendations (1)

### ■ Current Uses of *In Vitro* Cytotoxicity Testing by Industry

- Since large reductions in animal use have already been made for acute systemic toxicity testing, the impact of *in vitro* test methods on further animal reduction can be limited at best.
- *In vitro* test methods could eventually replace the *in vivo* acute toxicity test methods if a full battery of *in vitro* tests were available that take into account the many mechanisms and modes of action of acute toxicity.
- The availability of a validated *in vitro* test method for acute toxicity and the inclusion of such a test in a formal testing guideline would facilitate its widespread usage
  - However, industry tends to follow the most efficient track (i.e., use the standard *in vivo* test methods that regulatory agencies assuredly accept)
  - Industry is concerned about how the resulting data might be interpreted by regulators (i.e., may result in unfavorable regulatory actions)

# Breakout Group 5:

## Conclusions and Recommendations (2)

### ■ Submission of *In Vitro* and *In Vivo* Data to ICCVAM

- Noted that the current cost-benefit ratio doesn't justify using the validated *in vitro* methods to set starting doses because the number of animals used is already at a minimum
- Industry indicated willingness to provide data to ICCVAM in order to advance the development and validation of more predictive *in vitro* test methods.
  - However, they explicitly stated that guarantees (i.e., *in vitro* data would not be used for regulatory actions in the place of more favorable *in vivo* data) and incentives would likely be necessary
- Companies are likely to consider any mechanistic information to be proprietary
- Recommended creating a public/private consortium that would facilitate data collection and submission